# Hydrophobin HFBII production using fungal biofilm reactor and submerged bioreactor


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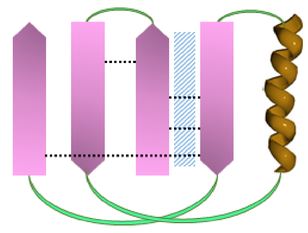
## Introduction

Hydrophobins are a novel family of low molecular weight proteins consisted of four disulfide bridges and an extraordinary hydrophobic patch[1]. They include exceptional surface active properties with real applications in several technological fields such as food and pharmaceutical industry. Therefore, production and purification of hydrophobin have recently been the subject of intensive research[2]. However, the yield of production needs to be further improved for a generic use of hydrophobins at industrial scale.



*Structure of HFBII. The blue pattern determines the hydrophobic chains. The black dash lines represent the disulfide bridges.*

## Material and Methods

### Cultivation medium

According to the physiological mechanisms observed during the screening phase, a bioreactor set up has been designed for the production of hydrophobin HFBII by cultivation of *Trichoderma reesei*.

*T. reesei*

The medium of cultivation was consisted the components listed as Table.

| Component | Amount (g/L) |
|---|---|
| Lactose | 40.0000 |
| Peptone | 4.0000 |
| Yeast Extract | 1.0000 |
| $KH_2PO_4$ | 4.0000 |
| $(NH_4)_2SO_4$ | 2.8000 |
| $MgSO_4.7H_2O$ | 0.6000 |
| $CaCl_2.2H_2O$ | 0.8000 |
| $FeO_4.7H_2O$ | 0.0100 |
| $CoCl_2.6H_2O$ | 0.0040 |
| $MnSO_4.H_2O$ | 0.0032 |
| $ZnSO_4.7H_2O$ | 0.0069 |

### Bioreactor set up

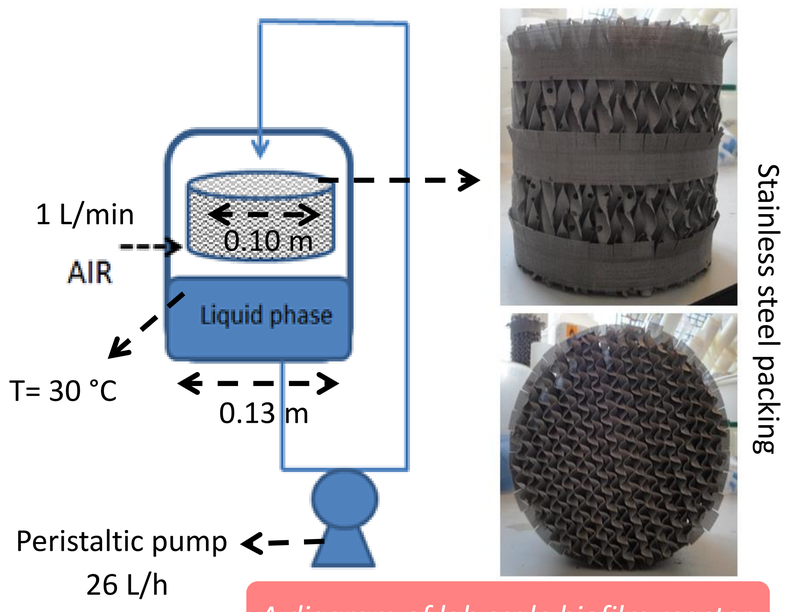
Two modes of cultures have been investigated to produce HFBII, *i.e.* the classical submerged fermentation and a fungal biofilm reactor.

### Submerged bioreactor

A first set of cultures were performed in a lab scale 1-L bioreactor. Submerged culture was carried out by using a stirring system with 6 blades running at 800 $min^{-1}$.

### Biofilm reactor

For biofilm reactor, the details are described in the following Figure.




*A diagram of lab scale biofilm reactor.*

### Analytical methods

#### Lactose consumption

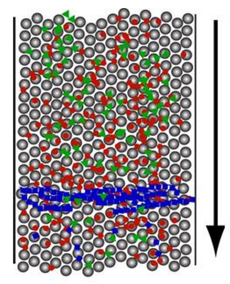
Lactose consumption during the fermentation was tracked by the enzymatic lactose assay kits.

#### Dry mass analysis

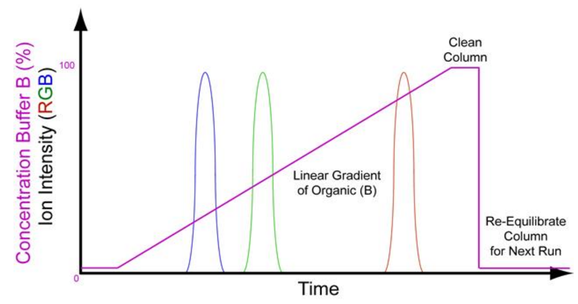
For determination of dry matter, 40 mL of the culture medium was filtered through a Whatman filter No.4, and dried in a Furnace at 105°C during 18 h.

#### Hydrophobin purification and determination

15RPC Liquid Chromatography was used for purification of HFBII. NanoDrop equipment was applied to quantify the purified proteins[3].



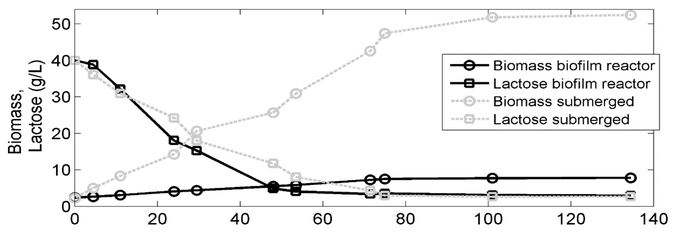
*The Overview on reversed phase chromatography for separation of different components.*




## Results and Discussion

### Carbon starvation

The major part of lactose has been consumed before the highest rate of fungal growth. Our results confirm that in both modes of reactors (*i.e.* submerged and biofilm) two criteria have to be met for the production of HFBII: excretion of extracellular enzymes for lactose hydrolysis and starvation for the induction of sporulation.

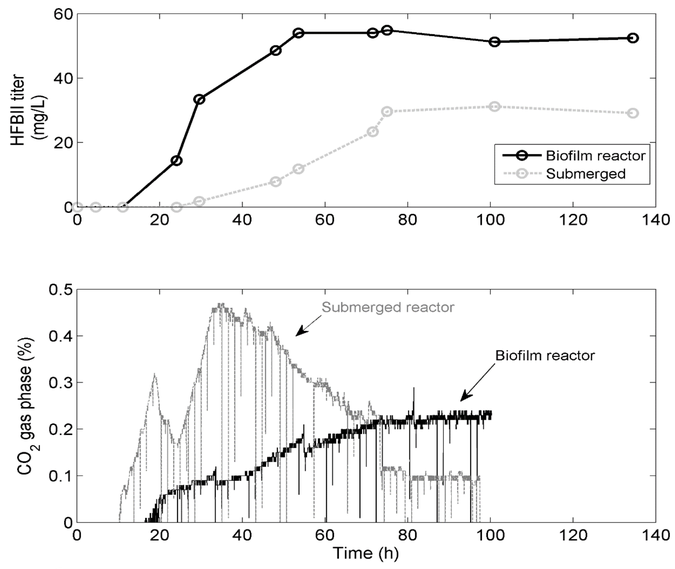



### Simplification of downstream processing

In our condition, the mode of biofilm reactor leads to the natural attachment of the main fungal biomass on the packing. The biomass in the liquid phase remained very low in compare with submerged reactor, allowing to alleviate the problem associated with the broth viscosity enrichment. This observation remarks the fact that biofilm reactor can be operated to simplify the downstream processing scheme, leading to a clear supernatant that can be directly treated for HFBII recovery without the need for prior centrifugation.

### Comparison between submerged bioreactor and biofilm reactor for HFBII production

The recorded $CO_2$ profiles show different trends in submerged and biofilm modes, suggesting that carbon is directed following altered metabolic pathways. One of the consequences of this carbon redirection is that HFBII is produced earlier and in higher amount in the biofilm reactor. This observation is in accordance with the physiology expected in biofilm reactor, *i.e.* a reduction of the time required to reach the secondary metabolisms with the excretion of the corresponding proteins and metabolites. More importantly, the fungal biomass attached onto the metal packing exhibit aerial structures promoting the production of hydrophobins.




*Comparative analysis of the hydrophobin HFBII production in submerged bioreactor and in biofilm reactor (n=3).*

## Conclusion

The production of HFBII occurs when the major part of the lactose has been consumed. This observation has led to set-up a biofilm reactor, promoting the growth of the fungal biomass in a solid state physiology, and the excretion of hydrophobin. The use of biofilm reactor has successfully led to 80% increase of HFBII production in shorter time by comparison with a classical submerged bioreactor.

## References


1. Deckers et al., 2012., J. Am. Soc. Brew. Chem. 70, 249–256.
2. Winterburn et al., 2011. Biochem. Eng. J. 54, 132–139.
3. Khalesi et al., 2013. Ind. Crop. Prod. 43, 372–377.